

Appl. No. 09/970,453
Amdt. dated October 26, 2004
Reply to Office action of April 26, 2004

AMENDMENT TO THE SPECIFICATION

Please amend the specification as follows:

Please INSERT before the first paragraph of the specification the following NEW paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/237,937, filed October 3, 2000, which is incorporated herein by reference in it entirety.

Please DELETE the second paragraph of the as-filed specification that reads:

~~CROSS-REFERENCE TO RELATED APPLICATIONS~~

~~—This application claims the benefit of U.S. Provisional Application No. 60/237,937, filed October 3, 2000, which is incorporated herein by reference in it entirety.~~

Please REPLACE the last paragraph on page 8 with the following amended paragraph:

While one can use multiple detectors and electromagnetic radiation sources (e.g., laser for laser induced fluorescence), it has been found by the present inventors that one or more, preferably two, acousto-optic modulators in conjunction with an aperture is particularly suitable for providing two different detection zones from a single laser source. An acousto-optic modulator is readily available from a variety of sources including at the world wide web at http://www.brimrose.com/acousto_modulators.html, which also includes a general discussion on the theory behind acousto-optic modulators (Brimrose Corp., Baltimore, Maryland). Other devices which is capable of guiding the laser beam into two or more different positions can also be used instead of an acousto-optic modulator. Such devices are well known to one of ordinary skill in the art and include rotating mirrors, gratings and other electromagnetic wave diffracting devices. The aperture allows emission of only one particular diffracted beam to illuminate the detection zones and blocks other diffracted laser beam.

Please REPLACE the last paragraph on page 15 with the following amended paragraph:

One method of achieving this correlation is to correlate the integrated peak area from the first detection zone to a corresponding integrated peak area in the second detection zone by limiting the detection to region of velocities about ± 4 times the median flow velocity of the fluid medium. For example, integrated peaks from the first detection zone is compared to integrated peaks from the second detection zones in the range of $1/4$ the median flow velocity to $4x$ the median flow velocity of the fluid medium. By comparing other integrated peak areas of similar intervals, one can verify whether the initial correlation is accurate. Such a method for detecting and correlating the integrated peak areas from the

first detection zone to the integrated peak areas of the second detection zone is schematically outlined in the flow sheets in Figures 7 and 8. Briefly, the two integrated peak areas (e.g., $A_l(i)$ and $A_r(i)$) are compared to see whether they are within the minimum and the maximum time difference as specified. The minimum and maximum time differences can be adjusted depending on the median flow velocity of the fluid medium. Once a pair of matching integrated peaks from the first and the second detection zones is found, the time difference (i.e., TimeDiff or Δt) is determined. The time difference is inversely proportional to the analyte flow velocity and is the difference in time when the particle crosses (i.e., detected by) the first and second detection zones. The velocity independent integrated peak area is then calculated by averaging the integrated peak areas of the particle from the first and the second detection zones and dividing the average integrated peak area by time difference. This calculation can be represented by the following formula:

$$A_{vi} = [A_l + A_r/2]\Delta t$$

where A_{vi} is velocity independent area, A_l is integrated peak area from the first detection zone, A_r is integrated peak area from the second detection zone and Δt is time difference. In Figure 8, the time difference is determined by dividing the difference in point number of A_l and A_r with the sampling rate (i.e., the frequency of switching the laser beam to and from the first and the second detection zones).